REMARKS

Claims 1-61 were pending prior to this response. By the present communication, claims 14, 21 and 27-61 have been cancelled without prejudice, no claims have been added, and claims 1, 15 and 24 have been amended to define Applicants' invention with greater particularity. The claim amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1-13, 15-20 and 22-26 are currently pending.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-26 under 35 U.S.C. § 112, first paragraph, for allegedly lacking sufficient disclosure to indicate that the inventors had possession of the broad genus claimed at the time of filing. In particular, Applicants disagree with the Examiner's assertion: "there is simply no common attributes that can link together all of the microenvironments, clones, probes and markers that should be screened and thus included in this enormous genus from the few examples provided by the applicants" (Office Action, page 6).

However, Applicants respectfully submit that the analogy of a method claim to a chemical compound (i.e., what atoms are included) is not apt. In these method claims, the framework that links together the terms in the claims is the syntax of English and the methodology (series of acts) of the claim in which they appear. Moreover, despite the Examiner's assertion that a single example of the invention is illustrated, Applicants submit that the Specification gives more than one and in some cases a plethora of specific examples illustrating the very terms identified by the Examiner as lacking requisite specificity to support the broad terms in the claims. As examples of "microenvironments" the Specification recites gel microdroplets (GMDs), beads, ghost red blood cells, macrophages and a variety of liposomes (page 23, lines 18 to page 24, line 18). Specific representative examples of "library clones" provided in the Specification are "gene expression libraries" (page 5, line 12), "nucleic acid libraries derived from DNA, for example DNA directly isolated from the environment" (page 15,

line 9), "a library of polyketides and postpolyketide biosynthesis genes for generation of novel polyketides" (page 32, lines 11-12), "polynucleotide library" (original claim 14). The generic term "expression vector" is described as "containing expression regulatory sequences which can control and regulate the production of a detectable protein or protein-related array activity" (page 32, lines 17-19 and numerous examples of specific vectors are provided, including "plasmids, phages, cosmids, phagemids, viruses (*e.g.,* retroviruses, parainfluenzavirus, herpesviruses, reoviruses, paramyxoviruses, and the like), or selected portions thereof (*e.g.,* coat protein, spike glycoprotein, capsid protein). For example, cosmids and phagemids are typically used where the specific nucleic acid sequence to be analyzed or modified is large because these vectors are able to stably propagate large polynucleotides" (page 35, lines 18-23) and f-factor vectors (page 36, lines 10-17), and lambda phage (page 36, lines 27-28).

Specific examples of a "mixed population of organisms" provided are indeed numerous: "insect feces, soil, water, *etc"*; "an extract from blood, urine, spinal fluid, tissue, vaginal swab, stool, amniotic fluid or buccal mouthwash" from any mammalian organism. For non-mammalian (*e.g.,* invertebrates) organisms the specific examples of mixed populations of organisms are "tissue samples, salivary samples, fecal material or material in the digestive tract of the organism". Such mixed populations of organisms are described as available in specific locations known to those of skill in the art, such as "hot sulfur pools, volcanic vents, and frozen tundra" in "plants, fertilizer, soil, liquid or other horticultural or agricultural product"; in "infant formula, seafood, fresh produce and packaged food"; in "soil, sewage treatment sludge; and in "blood, soil and sludge" (page 34, lines 7-24).

Specific examples of "markers" described in the Specification include the herpes simplex thymidine kinase gene" and "markers that can be selected using methods based on the detection of radioactivity, of enzymatic activity, of fluorescence, of any optical feature, of a magnetic

property (*e.g.*, using magnetic beads), of immunoreactivity, and of hybridization (page 72, lines 24-27).

The background recites numerous fluorescent markers used in flow cytometry, including "a range of fluorogenic esters including fluorescein diacetate (FDA) derivatives and CemChrome B" (page 9, lines 15-16).

Thus, Applicants submit that the Examiner's assertion that the Application fails to provide a representative number of examples illustrating the terms in the claims is erroneous. Moreover, Applicants submit that those of skill in the art would understand that the Applicants were "in possession" of the invention as claimed and would have understood their intention at filing of the application that the invention could be practiced in accordance with the full scope of the claims, rather than being limited to the particular example used to illustrate the invention in the Examples of the Specification. Accordingly, reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112, first paragraph, for allegedly lacking sufficient written description to support the claims are respectfully requested.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 7, 8, 19 and 25 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite; however the Examiner fails to identify any alleged indefiniteness in claims 7, 8, 19 and 25. Instead, the Office Action refers only to claim 22. Apparently the reference to claims 7, 8, 19 and 25 was in error and the following remarks are based on the assumption that only claim 22 is at issue. If this assumption is incorrect, Applicants' representative requests a telephone call from the Examiner to discuss the matter.

With regard to claim 22, the Examiner alleges that the phrase "small molecule" is a relative term, thus introducing lack of clarity into the claim. However, Applicants submit that

the phrase "small molecule" as used in Applicants' specification and claims does not refer specifically to the size of the molecule. Those of skill in the art would understand that, as used in claim 19, the phrase "small molecule" is used to distinguish a chemical molecule or complex, such as a non-proteinaceous enzyme, from molecules containing amino acids or nucleic acids, either of which may be smaller in terms of molecular weight than a large chemical complex. Since the definiteness of claim language is determined with reference to the understanding of those of skill in the art, and Applicants submit that those of skill in the art would readily understand the meaning of the phrase "small molecule" as used in claim 22, Applicants submit that claim 22 is definite as written. Therefore, Applicants respectfully submit that all claims meet the requirements for definiteness under 35 U.S.C. § 112, Second Paragraph and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection Under 35 U.S.C. § 102 (e)

Applicants respectfully traverse the rejection of claims 1-10, 12-20 and 22-26 under 35 U.S.C. § 102 (e) as allegedly being anticipated by Thompson et al. (U.S. Patent No. 5,824,485; hereinafter "Thompson"). Claim 14, has been cancelled, thereby rendering the rejection moot as to claim 14. Therefore, Applicants will address the rejection as to the currently presented claims.

Applicants respectfully submit that the invention methods for identifying a bioactivity or a biomolecule of interest, as defined by amended claim 1, distinguishes over the disclosure of Thompson by requiring:

(a) obtaining a plurality of polynucleotides derived from a mixed population of organisms or more than one organism;

(b) normalizing the plurality of polynucleotides;

(c) contacting a library containing clones of normalized polynucleotides from (b) with at least one oligonucleotide probe labeled with a detectable molecule; and

(d) separating clones with an analyzer that detects the detectable molecule.

Applicants describe "normalizing" and its advantages as follows in U.S. Patent 6,174,673, which is incorporated by reference into the present application:

> One embodiment for forming a normalized library from an environmental sample begins with the isolation of nucleic acid from the sample. This nucleic acid can then be fractionated prior to normalization to increase the chances of cloning DNA from minor species from the pool of organisms sampled. DNA can be fractionated using a density centrifugation technique, such as a cesium-chloride gradient. When an intercalating agent, such as bis-benzimide is employed to change the buoyant density of the nucleic acid, gradients will fractionate the DNA based on relative base content. Nucleic acid from multiple organisms can be separated in this manner, and this technique can be used to fractionate complex mixtures of genomes. This can be of particular value when working with complex environmental samples. . . This "normalization" approach reduces the redundancy of clones from abundant species and increases the representation of clones from rare species. These normalized libraries allow for greater screening efficiency resulting in the identification of cells encoding novel biological catalysts.

In the '673 patent, Applicants also teach: "single-stranded nucleic acid representing an enrichment of rare sequences is amplified using techniques well known in the art, such as a polymerase chain reaction (Bames, 1994), and used to generate gene libraries. This procedure leads to the amplification of rare or low abundance nucleic acid molecules, which are then used to generate a gene library which can be screened for a desired bioactivity."

Thompson is silent regarding "normalizing" polynucleotides, as the term is used in Applicants' specification and claims, to form a normalized library of DNA clones, which is then screened. Thompson's few comments regarding amplifying the copy numbers of a genomic library occur in two contexts. The first of these in Section 5.3.3. ENRICHMENT OF NON-RIBOSOMAL SEQUENCES FROM TOTAL RNA, concerns, as the title describes, separation of ribosomal sequences from total RNA. In the second, Section 5.4.8. PRE-SCREENING OF EXPRESSION LIBRARIES, three categories of pre-screening are described: "intracellular pre-

screening", which entails introduction of the library into a host engineered to contain a chemo-responsive reporter construct and selecting cells by fluorescence-activated cell sorting (FACS) or macrodroplet sorting; 2) differential pre-screening, which entails incubation of the library in the host with fluorescent or chromogenic physiological tracers, followed by FACS or macrodroplet sorting; and 3) "selection pre-screening," which entails incubation of the library in the host with selective agents such as antibiotics, followed by FACS or macrodroplet sorting to identify surviving or multiplying cells. For all these methods, cell sorting is done on bulk cultures of amplified libraries prior to examination of individual cultures. Thus, Applicants' goal for the normalization step is preparation of *naturally occurring molecules* for equal representation in a screening library followed by screening.

By contrast, to reduce the number of clones that need to be screened, Thompson describes pre-selection of DNA fragments for the screening library using probes and refers to this process as "biasing" a library. Such probes are described as being "prepared from known genes that may be related to or are involved in producing compounds of interest" (Thompson, Col 32, lines 6-7). However, rather than using the probes for screening (e.g., identifying molecules having a nucleotide sequence complementary to the probes) of a library of already "normalized" naturally occurring molecules, as in Applicants' claim 1, Thompson uses the activity probe concept for preparing "chimeric" and "biased" "*combinatorial expression libraries*" (See Thompson, Section 5.1.6) prior to screening.

Applicants provide extrinsic evidence in support of the meaning of "combinatorial" as used in Thompson to distinguish such teaching from Applicants' teaching and claims directed to screening of naturally occurring molecules. Exhibit "A" is a print out from an internet site that includes a description of Neugenesis' combinatorial biology technology, which creates "combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the

host by Neugenesis' proprietary technology." (http://www.neugenesis.com/) Clearly, Applicants' claims are not directed to combinatorial approaches to identifying enzyme activities encoded by naturally occurring gene clusters, since Applicants are not manipulating the DNA to generate variants.

Exhibit "B" is a printout from the internet site of the Koide Group, from University of Pittsburgh (http://www.pitt.edu/~sparano/group/). As you will note, the study of Natural Products is separate and distinct from the study of Combinatorial Libraries. Exhibit "C" provides a glossary of terms used in Medicinal Chemistry. On page 4, the term combinatorial synthesis is described as "...combining sets of building blocks" e.g., ligating together individual genes of a gene cluster.

Thus, in view of Thompson's use of "biased" libraries in the context of preparing combinatorial libraries, Applicants submit that Thompson neither discloses "normalization" of naturally occurring molecules as the term normalization is used in Applicants' claim 1, nor uses fluorescent probes for screening of such libraries. Accordingly, Applicants respectfully submit that Thompson fails to disclose each and every element of independent claim 1 (and dependent claims 2-10, 12-13, 15-20 and 22-26) as would be required to establish anticipation under 35 U.S.C. 102(e).

The Rejection under 35 U.S.C. § 103

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on

applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

Applicants respectfully traverse the rejection of claims 1-10, 12, 13, 15-20, and 22-26 under 35 U.S.C. § 103 as allegedly being unpatentable over Thompson (as above) and Miao et al, Biotechnology and Bioengineering (1993) 42:708-715, hereinafter "Miao". Applicants respectfully submit that the invention methods for identifying a bioactivity or a biomolecule of interest, as defined by amended claim 1, distinguishes over the combined disclosures of Thompson and Miao by requiring:

(a) obtaining a plurality of polynucleotides derived from a mixed population of organisms or more than one organism;

(b) normalizing the plurality of polynucleotides;

(c) contacting a library containing clones of normalized polynucleotides from (b) with at least one oligonucleotide probe labeled with a detectable molecule; and

(d) separating clones with an analyzer that detects the detectable molecule.

Thus, Applicants' invention, as defined by amended claim 1, is not the purposeful creation of novel activities or pathways by combinatorial techniques, but rather expression cloning of naturally occurring DNA derived from a mixed population of organisms to produce libraries of naturally occurring activities or gene clusters or pathways or genes as found in nature, without manipulation. In addition, polynucleotides in the libraries are equally represented (i.e., "normalized") on some basis selected to assure that those from organisms whose presence in the sample is rare on the selection basis, can have an equal chance that a naturally occurring "activity" encoded therein will be discovered as do organisms whose populations predominate in the sample. Thus the

polynucleotides in Applicants' library are both "normalized" and naturally occurring, meaning that the polynucleotides have not been rearranged or recombined in a laboratory setting for the purpose of creating new, combinatorially produced, pathways.

The deficiencies of Thompson described above for disclosing the invention methods apply equally and are incorporated here. In addition, Applicants respectfully submit that Thompson fails to suggest the invention methods and would not motivate those of skill in the art to modify Thompson to arrive at the presently presented invention methods because the thrust of Thompson's disclosure is devoted to preparation and screening of combinatorial gene libraries and Thompson's comments regarding preparation of "biased" libraries pertain specifically to the preparation of such combinatorial libraries. Specifically, Thompson's "biasing" technique does not suggest and would not motivate those of skill in the art to reduce the size of a collection of naturally occurring polynucleotides derived from a mixed population of organisms to increase the chances that an activity encoded by a rare organism in the sample will be as likely to be discovered in the screening as that of an organism whose presence predominates in the sample.

Applicants submit that the disclosure of Miao fails to remedy the deficiencies of Thompson under 35 U.S.C. § 103. Miao's disclosure pertains to use of C12FDG as a fluorescent substrate in FACS screening of single bacterial cells of one species (i.e., E. coli). Thus, like Thompson, Miao is completely silent regarding screening of a *normalized* library containing a plurality of clones obtained from a mixed population of organisms. Indeed, since Miao's disclosure does not pertain to screening of a plurality of species, as would be inherent in a "mixed population", Applicants submit that the combined disclosures of Thompson and Miao would be insufficient to motivate those of skill in the art to create a method for screening naturally occurring polynucleotides in which the chances of discovering a desired activity encoded by a rare organism in the sample are normalized with those of species in the sample that predominate.

In addition, even if those of skill in the art were motivated by the combined disclosures of Thompson and Miao to arrive at the invention methods, especially that of claim 12, Applicants submit that the cited art would fail to provide the reasonable expectation of success that is required to show unpatentability under 35 U.S.C. § 103. Because neither Thompson nor Miao discusses any technique by which a diverse library can be adjusted to provide equal representation of the polynucleotides obtained from rare members, those of skill in the art would not be justified in assuming success in the outcome of any technique that might be devised.

Accordingly, Applicants respectfully submit that the combined disclosures of Thompson and Miao, including Miao's disclosure regarding rapid screening using C12FDG, are not sufficient to teach or suggest Applicants' invention of dependent claim 12, which contains the requirements of amended claim 1, including the "normalization" of the polynucleotide pool used to make the library to be screened.

Thus, Applicants respectfully submit that claims 1-10, 12, 13, 15-20 and 22-26 are not *prima facie* obvious over Thompson, or the combined disclosures of Thompson and Miao. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 are respectfully requested.

The Double Patenting Rejection

Applicants respectfully traverse the rejection of claims 1-20 and 22-26 under the judicially created doctrine of obviousness type double patenting as being unpatentable over (U.S. Patent No. 6,174,673 to Short in view of Thompson. To overcome the rejection, Applicants submit herewith a Terminal Disclaimer disclaiming the terminal part of any patent granted on the subject matter of the above-identified Application and agreeing that any patent so granted on the above-identified application shall be enforceable only for and during such period that the legal title to the subject matter of said patent shall be the same as the legal title to U. S. Patent No.

6,174,673. In view of the Terminal Disclaimer submitted herewith, Applicants submit that U.S. Patent 6,174,673 is not available as prior art against this application.

Applicants further submit that Thompson alone is insufficient to disclose or suggest the invention methods for the reasons above stated. The deficiencies of Thompson described above for disclosing the invention methods apply equally and are incorporated here. In particular, Thompson fails to suggest the invention methods and would not motivate those of skill in the art to modify Thompson to arrive at the presently presented invention methods because the thrust of Thompson's disclosure is devoted to preparation and screening of combinatorial gene libraries. Furthermore, Thompson's comments regarding preparation of "biased" libraries pertain, not to "normalizing" a collection of polynucleotides as the term is used in Applicants' application and claims, but refer to preparation of *combinatorial* libraries. Specifically, Thompson's "biasing" technique does not suggest and would not motivate those of skill in the art to reduce the size of a collection of naturally occurring polynucleotides derived from a mixed population of organisms to increase the chances that an activity encoded by a rare organism in the sample will be as likely to be discovered in the screening as that of an organism whose presence predominates in the sample.

In addition, Applicants respectfully traverse the rejection of claims 1-20 and 22-26 under the judicially created doctrine of obviousness type double patenting as being unpatentable over U.S. Patent Application No. 2001/0041333 A1 (Serial No. 09/738,871). To overcome the rejection, Applicants submit herewith a Terminal Disclaimer disclaiming the terminal part of any patent granted on the subject matter of the above-identified Application and agreeing that any patent so granted on the above-identified application shall be enforceable only for and during such period that the legal title to the subject matter of said patent shall be the same as the legal title to any patent that may be granted on U.S. Patent Application No. 2001/0041333 A1. In

view of the Terminal Disclaimer submitted herewith, Applicants submit that U.S. Patent Application No. 2001/0041333 A1 is not available as prior art against this application.

In view of the Terminal Disclaimer submitted herewith and the above remarks, Applicants respectfully request reconsideration and withdrawal of the rejection for obviousness type double patenting.

CONCLUSION

In summary, in view of the Terminal Disclaimer and for the reasons set forth herein, Applicants maintain that claims 1-10, 12, 13, 15-20 and 22-26 clearly and patentably define the invention and respectfully request that the Examiner withdraw all rejections and pass the

application to allowance. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Terminal Disclaimer
Exhibit "A"
Exhibit "B"
Exhibit "C"